ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2006, p. 756–758 Vol. 50, No. 2
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.2.756–758.2006

Occurrence of OXA-58-Like Carbapenemases in *Acinetobacter* spp. Collected over 10 Years in Three Continents

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Received 20 September 2005/Returned for modification 24 October 2005/Accepted 30 October 2005

OXA-58 is a recently described carbapenemase from *Acinetobacter* spp. in Europe. We examined earlier worldwide *Acinetobacter* collections and found bla_{OXA-58} in 30 carbapenem-nonsusceptible isolates, including several isolates collected in Argentina and Kuwait in 1995 and 1996 and in a British outbreak strain from 2000. Most isolates (28 of 30) also had bla_{OXA-51}. We conclude that bla_{OXA-58} is geographically widespread and has occurred in *Acinetobacter* spp. for over 10 years.

Acinetobacter spp. are opportunistic pathogens that can survive for long periods in the hospital environment. Most *Acinetobacter* infections are caused by *Acinetobacter baumannii*, a species with frequent multiresistance to antibiotics other than carbapenems and polymyxins. Carbapenems are the standard therapy for severe *Acinetobacter* infections, and it is disturbing that *A. baumannii* isolates with resistance or reduced susceptibility are increasingly reported (3, 14). Some of these, particularly those in the Far East, have IMP and VIM metalloenzymes (15); but more isolates, particularly those in Europe, have OXA carbapenemases (18). These divide into four clusters by sequence homology, comprising OXA-23, OXA-24, OXA-51, and OXA-58, respectively, and their sequence variants.

OXA-23-like enzymes have been found since 1985 in the United Kingdom, East Asia, and South America (2, 6, 8, 24), whereas OXA-24-like enzymes, found since 1996, mostly occur in isolates epidemiologically linked to Spain and Portugal (7, 16). OXA-51 and OXA-58 are the most recent discoveries. OXA-51-like enzymes have carbapenemase activity in vitro (4, 5) but are very widespread in *A. baumannii*, including carbapenem-susceptible strains (12, 23). They may confer resistance only in particular circumstances, which have yet to be defined fully. OXA-58 is much less common and, like OXA-23 and OXA-24, is more consistently associated with nonsusceptibility, as also confirmed by transfer and inactivation studies (11, 13, 20).

The first known OXA-58-producing *Acinetobacter* isolate was collected in France in 2003 (19). Subsequently, this enzyme was found in an outbreak strain in a French hospital (11) and in isolates from several southern and eastern European countries (17). These results, along with OXA-58's proven role in resistance, prompted us to reexamine carbapenem-resistant acinetobacters and those with diminished susceptibilities in which we had previously failed to define a resistance mechanism. These isolates were collected worldwide over the past 10 years. They comprised 49 isolates from a United Kingdom survey conducted in 2000 (10), 185 United Kingdom reference submissions from 2002 or later, 3 recent reference submissions from Austria, and 7 isolates from Argentina and Kuwait (2). All required imipenem and/or meropenem MICs $\geq$1 μg/ml, compared with modal MICs of 0.25 μg/ml for *Acinetobacter* spp. The Argentinean and Kuwaiti isolates dated from 1995 and 1996 and were inferred to have OXA-type carbapenemases, based on the more rapid hydrolysis of oxacillin than benzylpenicillin in spectrophotometric assays, although attempts to clone and sequence these enzymes were unsuccessful (1, 2).

Identification to the genospecies level was by DNA fingerprinting for the United Kingdom and Austrian isolates (9, 10) or amplified ribosomal DNA restriction analysis (22) for the Argentinean and Kuwaiti isolates (1, 2). The isolates were typed by pulsed-field gel electrophoresis (PFGE) of ApaI-digested DNA by use of the extraction and analysis methods of Turton et al. (21). Alleles for bla_{OXA-51}-like genes were sought by PCR with primers OXA-51F (TAATGCTTTGATCGGCC TTG) (4, 5) and OXA-51R (TGGATTGCACTTCATCT TGG) (S. Brown, personal communication), while bla_{OXA-58}-like alleles were sought with primers OXA-58F1 (ATGAAAT TATTAAAAATATTGAGTTTAGTTTGC) and OXA-58R1 (TTATAAATAATGAAAAACACCCAACTTATC). The following conditions were used in both PCR systems: 94°C for 6 min; 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min; and finally, an extension at 72°C for 5 min. The PCR products were cleaned by using a Hybaid DNA Recovery Kit II (Hybaid, Ashford, United Kingdom), and both strands were sequenced with a CEQ DTCS-Quick Start kit (Beckman Coulter, High Wycombe, United Kingdom). Contigs were assembled by using GeneBuilder (Applied Maths, Sint-Martens-Latem, Belgium) and BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) software.

A total of 30 isolates, all belonging to the *A. baumannii-A. calcoaceticus* complex, had bla_{OXA-58} alleles (Fig. 1). These comprised 14 carbapenem-resistant isolates (defined on the

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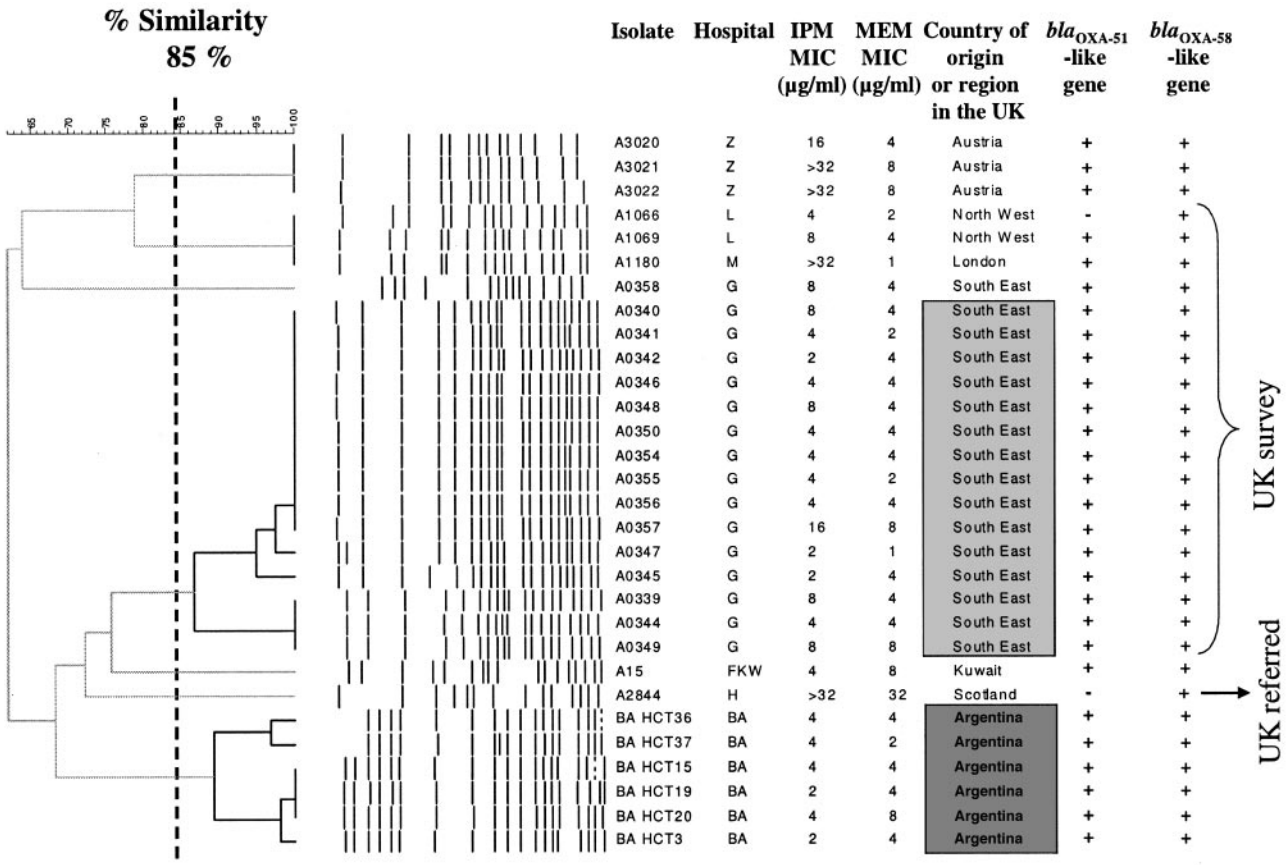

FIG. 1. Characteristics and sources of *Acinetobacter* sp. isolates with bla_{OXA-58}. Clonal isolates from the cluster of 15 isolates collected at a British hospital in 2000 are shaded light gray; those collected from the hospital in Argentina in 1995 and 1996 are shaded dark gray. "UK referred" means referred to the national reference laboratory from diagnostic laboratories within the United Kingdom. IPM, imipenem; MEM, meropenem.

basis of British Society for Antimicrobial Chemotherapy criteria of an MIC of >4 μg/ml) and 16 isolates with reduced carbapenem susceptibility (MICs, 1 to 4 μg/ml). Twenty-eight of these 30 isolates also had bla_{OXA-51}-like alleles (Fig. 1), as did many of the other *Acinetobacter* sp. isolates tested, supporting the view that bla_{OXA-51}-like alleles are widespread in *Acinetobacter* spp., whether these are carbapenem nonsusceptible or not (12, 23). The isolates with bla_{OXA-58} included *A. baumannii* BAHCT3, BAHCT15, and BAHCT19 (1), along with a further three isolates (isolates BAHCT3, BAHCT36, and BAHCT37), all of which belonged to the same PFGE-defined clone and all of which were collected at the same hospital in Buenos Aires, Argentina, in 1995; bla_{OXA-58} was also found in *A. baumannii* isolate A15 (NCTC 13305), which was collected in Kuwait in 1996 (2). These seven isolates, all of which also had bla_{OXA-51}-like enzymes, now represent the earliest-known OXA-58 producers. bla_{OXA-58}- and bla_{OXA-51}-like carbapenemase genes were also consistently present together in the members of a clonal cluster of 15 isolates collected from a hospital in southeast England between January and October 2000 during a national survey (10); isolates belonging to this clone are shaded light gray in Fig. 1. Both carbapenemase genes were present in three further isolates (isolates A1069, A1180, and A0358) collected from separate United Kingdom hospitals in the 2000 survey. Even though they were from geographically remote hospitals that were unlikely to transfer patients to one another, the first two of these three isolates appeared to be related by PFGE. Finally, both bla_{OXA-58}- and bla_{OXA-51}-like carbapenemase genes were also present together in the clonal group of three isolates (isolates A3020, A3021, and A3022) collected at one hospital in Austria in 2003. bla_{OXA-58}—but not bla_{OXA-51}—was present in two further United Kingdom isolates; one of them (isolate A2844) was unique by PFGE, and the other one (isolate A1066) was apparently related to two isolates (isolates A1069 and A1180) that had both bla_{OXA-58} and bla_{OXA-51}. PFGE revealed no clonal relationships between bla_{OXA-58}-positive isolates from different countries.

With one minor exception, the sequences obtained for OXA-58-like amplicons from all 30 gene-positive isolates were identical to the original bla_{OXA-58} sequence (GenBank accession number AY570763) between nucleotides 53 and 797. This portion represents 88% of the complete gene. The exception was isolate BAHCT19, collected in Argentina in 1995, with a silent T-to-C mutation at position 742.

The carbapenem MICs for the bla_{OXA-58} producers varied from 32 μg/ml for members of the Austrian strain to 1 to 4 μg/ml for the Argentinean isolates and for some of those from the United Kingdom survey (Fig. 1). It may be significant that the carbapenem MICs for the earlier isolates tended to be

lower than those for the more recent isolates. In any event, the relatively low MICs for some producers imply that these organisms might spread undetected. The reasons for variation in the levels of resistance are unknown but may reflect the level of expression of the OXA-58 enzyme or the OXA-51 enzyme, or both, along with permeability and efflux factors.

These data support the view that the OXA-58 carbapenemase, like the enzymes in the OXA-23 group, is globally scattered among *Acinetobacter* isolates, sometimes occurring in outbreak strains, and show that it reached the genus at least 10 years ago. Its origin, like those of the OXA-23 and the OXA-24 enzymes, remains uncertain, although it is notable that all these OXA carbapenemases have G+C ratios (34 to 39%) typical of those for the *Acinetobacter* genome as a whole (39 to 47%) but different from that of *Pseudomonas aeruginosa*, where OXA-40 (OXA-24 related) has also been recorded (E. Sevillano, M. J. Canduela, A. Umaran, F. Calvo, and L. Gallego, Abstr. 14th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P689, 2004). Such data are consistent with but do not prove a long evolutionary history in the genus (15). Whatever their origin, the spread of the OXA carbapenemases in *Acinetobacter* spp. is a growing problem: many producer strains are resistant not only to carbapenems but also to all other antibiotics except polymyxins and, perhaps, tigecycline.

(This work was presented in part at the 6th International Symposium on the Biology of *Acinetobacter*, Dublin, Ireland, 15 to 17 September 2004.)

The work of J.C. on carbapenem resistance in *Acinetobacter* spp. was supported by AstraZeneca. The work of N.W. and D.L. on emerging β-lactamases is supported, in part, by the EU/FP6-funded COBRA project (6-PCRD LSHM-CT-2003-503-335).

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